Standard Operating Procedure For The Determination of Carbamates in Water by High Performance Liquid Chromatography

1.0 Purpose

1.1 The purpose of this SOP is to allow for qualitative and quantitative determination of carbamate insecticides that may be present in water.

2.0 Scope

2.1 The carbamate analysis is done by utilizing High Performance Liquid Chromatography(HPLC). This SOP is modeled after EPA Method 531.1(1). The carbamates are separated by the HPLC column. They are then hydrolyzed by a basic solution followed by a reaction with a complex solution to form a derivative that is detected by a fluorescence detector. This SOP is applicable to water samples such as drinking water, ground water, and surface water. The low limit of quantitation is currently 0.5 μg/L. Carbamates that can be analyzed by this method include(but not limited to) are:

Analyte (LIMS #)	CAS #	MDL(ug/L)
Aldicarb (Analyte number 30250)	116-06-3	0.629
Aldicarb Sulfone (30295)	1646-88-4	0.444
Aldicarb Sulfoxide (30290)	1646-87-4	0.567
Carbaryl (30950)	63-25-2	0.370
Carbofuran (30535)	1563-66-2	0.419
3-Hydroxycarbofuran (30560)	16655-82-6	0.397
Methomyl (30565)	16752-77-5	0.370
Oxamyl (30300)	23135-22-0	0.469

Method detection limits are calculated using the following:

MDL = S t(n-1, 1-alpha=0.99)

Where:

t(n-1,1-alpha = 0.99) = Student's t value for the 99% confidence level with n-1 degrees of freedom

N = number of replicates

S = standard deviation of the replicate analyses.

MDL's calculated based on entered LIMS results from 01/01/02 - 01/01/03 using LFB4.0 results.

3.0 Safety

3.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. Material safety data sheets (MSDS's) should be on file for all analytes and reagents. One reagent in particular, 2-mercaptoethanol, has an offensive odor and is considered highly toxic. Special care should be taken when working with this reagent to minimize exposure.

4.0 Interferences

4.1 Method interferences may be caused be contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. The method must be demonstrated to be free from interferences by running laboratory reagent blanks with each analysis. Two specific sources of interferences are listed below.

4.1.1 Glassware must be scrupulously cleaned. Wash the glassware with water and detergent followed by rinsing with distilled water. Allow the glassware to dry and then rinse with acetone followed by hexane.

4.1.2 The use of high purity reagents and solvents helps to minimize interferences. The use of a linear gradient during analysis helps to minimize elevated baselines in the liquid chromatograms.

5.0 Sample Collection, Preservation and Handling

5.1 Sampling equipment and procedure

5.1.1 Grab sample bottle - 40 ml glass screw cap vials and caps equipped with PTFE-faced (Teflon faced) silicone septa. Prior to use, wash vials and septa as described in Section 4.1.1.

5.1.2 Collection procedure - Conventional sampling practices(2) should be followed; however, the bottle must not be pre-rinsed with sample before collection.

5.2 Sample Preservation/pH Adjustment - Aldicarb sulfoxide, carbaryl, 3-hydroxycarbofuran, and oxamyl can all degrade quickly in neutral and basic waters held at room temperature (3,4). This short term degradation is of concern during the time samples are being shipped and the time processed samples are held at room temperature in autosampler trays. All samples must be preserved at pH 3. The pH adjustment also minimizes analyte biodegradation.

5.2.1 If residual chlorine is suspected to be present, add 3.2 mg of sodium thiosulfate per 40 ml of sample to the sample bottle prior to collecting the sample.

5.2.2 A commercially prepared buffer, ChlorAC Buffer, for preservation is available from Pickering Laboratories. ChlorAC Buffer is ready-to-use and prepared to EPA specifications. It is guaranteed to be free of co-eluting

interferences for the carbamate analytes.

5.2.2.1 Add 1.2 ml ChlorAC Buffer to the 40 ml sample bottle. The buffer may be added to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site.

5.2.3 Alternative buffer - Add 1.2 ml monochloroacetic acid buffer (see Section 6.1.11 for preparation) to the 40 ml sample bottle. The buffer may be added to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site. We do not routinely use this procedure.

5.3 Sample Handling

5.3.1 After the sample has been collected in the container, seal the sample bottle with the Teflon-faced side of the septum toward the sample. Shake the sample for 1 minute.

5.3.2 Samples must be iced or refrigerated at 4 degrees C from the time of collection until storage.

5.3.3 Due to variances in sample matrices, the supplied buffer may or may not be adequate. The pH of the samples must be checked when the samples arrive at the laboratory. If necessary, make the appropriate pH adjustments with dilute hydrochloric acid or dilute sodium hydroxide. A 1/25 dilution of the concentrated acid and base should make adequate solutions for the pH adjustment.

5.3.4 Sample and blank spikes should be prepared when the samples arrive at the laboratory. They should be stored and analyzed with the samples. This will monitor for analyte degradation between when the samples are stored and analyzed.

5.3.5 Samples must be stored at -10 degrees C. Studies have indicated that the analytes are stable in water for at least 28 days when adjusted to pH 3 and stored at -10 degrees C. The procedure described in Section 5.3.4 will verify analyte stability in the sample matrix.

6.0 Chemicals, reagents and stock solutions

6.1 Chemicals

6.1.1 Reagent water - For use in the HPLC mobile phase and in the preparation of other reagents. Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Distilled water purified by a commercial system such as a Barnstead Nanopure II system is suitable for this procedure. The water from the Barnstead system must be filtered through 0.45 μM filters using mechanical means to remove small particles that are detrimental to the HPLC equipment.

6.1.2 Acetonitrile (CH_3CN) - HPLC grade acetonitrile for use in the HPLC mobile phase and in the preparation of reagents.

6.1.3 Methanol (CH_3OH) - HPLC grade methanol for use in the HPLC mobile phase and in the preparation of reagents.

6.1.4 Concentrated hydrochloric acid (HCl)

6.1.4.1 Diluted about 1/10 and used to adjust pH of reagent water to 3 for use in the preparation of standards and blanks.

6.1.4.2 Diluted about 1/25 and used to adjust pH of samples to 3 when they arrive at the lab, if necessary.

6.1.5 Concentrated sodium hydroxide (NaOH) - Diluted about 1/25 and used to adjust the pH of samples to 3 when they arrive at the lab, if necessary.

6.1.6 Sodium hydroxide pellets - for use in the preparation of post column reagents. Available from several sources.

6.1.7 Sodium borate ($Na_2B_4O_7 \cdot 10H_2O$), crystalline - For use in the preparation of post column reagents. Available from Fisher.

6.1.8 Ortho-phthalaldehyde (OPA) - For use in the preparation of the post column reaction solution. Available from Pickering. Also known as phthalic dicarboxaldehyde which is available from Aldrich.

6.1.9 2-Mercaptoethanol - For use in the preparation of the post column reaction solution. The compound has an offensive odor and is considered highly toxic.

6.1.10 4-Bromo-3,5-dimethylphenyl N-methylcarbamate (BDMC) - for use as an internal standard if desired. Available from Aldrich.

6.1.11 Monochloroacetic acid - For use in preparation of buffer for the pH adjustment of samples. Prepare buffer by mixing 156 ml of 2.5 M monochloroacetic acid and 100 ml 2.5 M potassium acetate. We do not routinely use this procedure, but it is included here for reference. Also know as chloroacetic acid. Available from Sigma.

6.1.12 Concentrated nitric acid - A 20% solution of nitric acid is used to rinse the post column system of deposits before shutting the system down.

6.2 Post-column derivatization solutions

6.2.1 2-Mercaptoethanol (1+1) - Mix 10.0 ml of 2-mercaptoethanol and 10 ml of acetonitrile. Do this procedure in a fume hood. Cap tightly and store the solution in a hood.

6.2.2 0.05 N sodium borate - Dissolve 19.1 g sodium borate in 1 L reagent water. Stir on a mechanical stirrer until dissolved.

6.2.3 0.05 N sodium hydroxide - This is the hydrolyzation solution. Dissolve 2.0 g sodium hydroxide in 1 L reagent water. Pass through 0.45 μM filter before use.

6.2.4 OPA reaction solution - This is the reaction solution. Dissolve 100 $\pm$ 10 mg of OPA in about 10 ml methanol. Add to 1 L 0.05 N sodium borate solution. Rinse the OPA container with about 2 ml methanol and add this to the borate solution also. IMPORTANT: This solution must be filtered through 0.45 μM filters before use. This will require several filter changes. The sodium borate solution will plug filters on the HPLC equipment if not pre-filtered. After filtering, add 100 μl 2-mercaptoethanol (1+1) and mix. Do not filter or degas after adding the 2-mercaptoethanol(1+1). This solution should be made fresh daily.

6.3 Spike solution preparation

6.3.1 Prepare a stock solution of the carbamate analytes at 400 ng/ml (400 ppb) in acetonitrile. Certified standard solutions or neat standards are available from several sources. Dilutions to reach 400 ng/ml will vary depending upon the concentration of the standard solutions. This solution should be stored at -10 degrees C. This solution can be used as long as QA/QC criteria are being

satisfied.

6.3.2 Lab fortified blank and lab fortified sample preparation.

The lab must analyze one lab fortified blank and one fortified sample with every 20 samples or one per sample set, whichever is greater. This should be done as each sample set arrives for analysis.

6.3.2.1 Using glass pipets, add 5 ml pH 3 reagent water and 5 ml sample to separate scintillation vials. Using an Eppendorf pipet, remove 50 μl from each of the vials. To each vial, add 50 μl of the 400 ng/ml stock solution prepared in Section 6.3.1.

400 ng/ml X 50/5000 = 4 ng/ml

Mix the solutions well. Store and analyze these solutions with the samples.

6.4 Stock standard solution preparation

6.4.1 Prepare a stock solution of the carbamate analytes at 200 ng/ml (200 ppb) in acetonitrile. Certified standard solutions or neat standards are available from several sources. Dilutions to reach 200 ng/ml will vary depending upon the concentration of the standard solutions. This stock solution should be stored at -10 degrees C. This solution can be used as long as QA/QC criteria are being satisfied. See Section 8.3.1 for preparation of the calibration solutions.

6.4.2 Prepare a stock solution of the internal standard at 1000 ng/ml (1000 ppb) in acetonitrile. Certified standard solutions or neat standards are available from several sources. Dilutions to reach 1000 ng/ml will vary depending upon the concentration of the standard solutions. This stock solution should be stored at -10 degrees C. This solution can be used as long as QA/QC criteria are being satisfied. See Section 8.3.2 for preparation of the internal standard in the calibration standards and the samples.

7.0 Equipment and apparatus

7.1 Analytical equipment

7.1.1 Varian 9010 HPLC pump. The pump is programmed at the instrument.

7.1.2 TSP AS3000. The autosampler is programmed at the instrument.

7.1.3 TSP FL3000 fluorescence detector or TSP FL2000 fluorescence detector. The detector is programmed at the instrument.

7.1.4 Pickering PCX 5100 Post Column Reaction Module

7.2 Miscellaneous apparatus

7.2.1 System for filtering of liquids before use on the HPLC system. This would include a vacuum source, appropriate filtration flasks, and appropriate reservoir for the liquid. The filters must be designed for the filtering of primarily aqueous solutions.

7.2.2 Helium gas for the purging and overlaying of the post column reagents to minimize reagent degradation. Helium gas is also used to sparge the solvents used in the mobile phase to prevent air bubbles in the flowcell of the detector.

7.2.3 Sample filters - Samples must be filtered to remove particulates before analysis. Filters are available from Whatman (Puradisc 25 TF, 0.45 μM pore size) or Gelman (Acrodisc 3, 0.45 μM pore size).

7.2.4 Disposable syringes to be used when filtering samples.

8.0 Procedure

8.1 Analytical conditions

8.1.1 HPLC column - MetaChem Technologies Inc., Inertsil C8-3 5 um, part number 0404-150X046, or any other columm that gives satisfactory results.

8.1.2 HPLC guard column - A guard column is not used. However, an in-line column pre-filter is used to protect the column from particulate contamination. The pre-filter is available from Alltech, stock number 28689.

8.1.3 HPLC mobile phase gradient

A: reagent water
B: methanol
C: acetonitrile

Time	%A	%B	%C
0	88	12	0
4	88	12	0
20	30	35	35
25	30	35	35
30	88	12	0
45	88	12	0

8.1.4 Flow: 1.2 ml/min

8.1.5 Method run time: 45 minutes

8.1.6 Data collection time: 20 minutes

8.1.7 Autosampler parameters:

Injection volume: 500 μl
Run time: 42 minutes

All other parameters can remain at default values.

If the mobile phase is significantly changed from the previous analysis, the syringe on the autosampler must be primed with the new mobile phase before use. See the Alcott 738 manual, section 6.4.2 for the procedure.

8.1.8 Detector parameters:

Excitation wavelength: 330 nm
Emission wavelength: 464 nm
Run time: 20 minutes

All other parameters can remain at default values.

8.1.9 Post column reaction system parameters:

Reagent 1 - 0.05 N sodium hydroxide solution (Section 6.2.3).
Reagent 2 - 2-mercaptoethanol and OPA in sodium borate solution (Section 6.2.4)
Flows: Approximately 0.3 ml/min.
HPLC column temperature: 30 degrees C
Reaction temperature: 80 degrees C

8.2 System setup and equilibration

8.2.1 The analytical HPLC system must be setup first. Always have the analytical pump operating before turning on the post column flows. The post column system will not function without pressure on the system from the analytical pump. This is a built in safety feature to prevent post column reagents from back flowing into the HPLC column.

8.2.2 Once the analytical pump is operating, prime the post column pump with the reagents. Allow this to equilibrate for 30 minutes.

8.3 Standard preparation

8.3.1 Using the 200 ng/ml stock solution of analytes prepared in Section 6.4.1., prepare standards for calibration of the system using the dilutions listed below.

Standard 1 200 ng/ml X 25 μl/10000 μl = 0.5 ng/ml

Standard 2 200 ng/ml X 50 μl/10000 μl = 1.0 ng/ml

Standard 3 200 ng/ml X 100 μl/10000 μl = 2.0 ng/ml

Standard 4 200 ng/ml X 300 μl/10000 μl = 6.0 ng/ml

The standards should be injected at the beginning of the analysis. If a large number of samples is to be analyzed, standards may be injected at the end of the analysis, also.

8.3.2 Using the 1000 ng/ml stock solution of analytes prepared in Section 6.4.2., prepare each standard and sample with a known constant amount of BDMC. Dilutions are listed below.

To each standard :

1000 ng/ml X 50 μl/10000 μl = 5.0 ng/ml

To each sample:

1000 ng/ml X 25 μl /5000 μl = 5.0 ng/ml`

8.4 Sample Preparation

8.4.1 The samples have a 28 day hold time. The samples should have had their pH checked when they arrived at the lab. A lab fortified blank and a fortified sample should have been prepared and stored with the samples. The samples are filtered through the HPLC filters mentioned in Section 7.2.3.

8.5 Spike Preparation

8.5.1 The preparation of the laboratory fortified blanks and fortified samples should be done when the samples arrive at the laboratory. See Section 6.3 for procedure. The spikes are filtered through the HPLC filters mentioned in Section 7.2.3

8.6 Computer setup

Computer setup and use of software will not be included in this SOP. The basics of the software will be provided here. Data analysis on XChrom consists of two main parts. They are the method file and the analysis file. The method file contains the acquisition parameters of the analytical data, the calibration parameters, and the integration parameters . The analysis file contains the sample order and other information such as sample type, the method utilized, and the concentration of internal standard.

8.7 System shutdown - Several steps must be taken after the analysis is complete to shut the analytical and post column systems down.

8.7.1 Analytical HPLC shutdown

8.7.1.1 When the analysis is complete, the Varian 9010 pump can be controlled from the pump keypads by pushing the remote button.

8.7.1.2 Pump 90/10 acetonitrile/water through the system for at least 20 column volumes which would be approximately 50 ml. This will remove any strongly adsorbed compounds from the system.

8.7.1.3 Pump 50/50 acetonitrile/water for 5 minutes. This will ease the mobile phase transition in the next step. Changing mobile phases to quickly can cause damage to the stationary phase.

8.7.1.4 Pump 90/10 water/acetonitrile for at least 20 column volumes. This will remove any polar compounds that may have accumulated in the system.

8.7.1.5 Equilibrate the system with 50/50 acetonitrile/water or 50/50 methanol/water. Pump at least 50 column volumes through the column. The column may be stored with this mobile phase.

8.7.2 Post column system shutdown - The analytical pump must always be flowing while the post column system is pumping. The post column system will shut down if there is no back pressure from the HPLC. This prevents post column reagents from backflowing into the analytical column.

8.7.2.1 When the analysis is complete, replace the post column reagents with reagent water and pump for about 15 minutes.

8.7.2.2 Replace the water with a solution of 20% nitric acid. The post column system is very sensitive to reagents plugging filters and causing high reagent pressures. Pickering recommends this step to minimize this effect. Pump the nitric acid solution for about 1 hour. Pump the nitric acid solution through the post column system only. Do not pump through the analytical pump.

8.7.2.3 Replace the 20% nitric acid solution with reagent water and pump for about 15 minutes.

8.7.2.4 Replace the water with 50/50 methanol/water and pump for an extended period of time, preferably overnight. Leave the reaction temperature at 80 degrees C. This will help any deposits present dissolve.

8.7.2.5 Set the reaction temperature and column temperature to 20 degrees C and allow the system to cool. This will take several hours. The reagent flows can be shut off after the system has cooled slightly.

The 50/50 methanol/water can remain in the system while not in use.

9.0 Quality Control

9.1 Quality control consists of the analysis of laboratory reagent blanks, laboratory fortified blanks and laboratory fortified samples.

9.1.1 Laboratory reagent blanks - A laboratory reagent blank (lab blank) must be analyzed with each set of samples. If within the retention time window of any analyte of interest the lab blank produces a peak that would prevent the determination of that analyte, determine the source of the contamination and eliminate the interference before processing samples.

9.1.2 Laboratory fortified blanks - The analyst must analyze at least one laboratory fortified blank (blank spike) with every 20 samples or one per sample set, whichever is greater. The spiking level should be 10 times the method detection limit, which is currently 0.5 μg/L. The spikes should be prepared when the samples arrive at the laboratory. See Section 6.3 for blank spike preparation.

9.1.2.1 Control limits - Control limits are determined by calculating upper and lower limits from the mean percent recovery (X) and the standard deviation (S) of the percent recovery. The equations for determining the limits are:

$$\text{Upper limit} = X + 3S$$
$$\text{Lower limit} = X - 3S$$

After each ten new recovery measurements, new control limits should be calculated using the most recent 30 data points.

9.1.2.2 Acceptability of results - Any analytes whose percent recovery in the blank spike for that set of samples is outside the control limits cannot be reported. The set must be reanalyzed with a new spike or not reported.

9.1.3 Laboratory fortified samples - The analyst must analyze at least one laboratory fortified sample (sample spike) for every 20 samples or one per sample set, whichever is greater. The spiking level should be approxiamately 10 times the method detection limit, which is currently approxiamately 0.5 μg/L. The spikes should be prepared when the samples arrive at the laboratory. See Section 6.3 for sample spike preparation procedure.

9.1.3.1 Control limits - Control limits are determined by calculating upper and lower limits from the mean percent recovery (X) and the standard deviation (S) of the percent recovery. The percent recovery should be corrected for any background concentration in the non-spiked sample, if necessary.

$$\text{Upper limit} = X + 3S$$
$$\text{Lower limit} = X - 3S$$

After each ten new recovery measurements, new control limits should be calculated using the most recent 30 data points.

9.1.3.2 Acceptability of results - Any analytes whose percent recovery in the sample spike for that set of samples is outside the control limits cannot be reported. The set must be reanalyzed with a new sample spike or not reported.

9.2 System suitability - No formal system suitability is done on a daily basis. The first standards are evaluated as they become available. Generally, if analyte retention times are similar to the previous analysis, peak shape is good, and baseline resolution is achieved between the early eluting analytes the system is considered suitable for analysis. HPLC system pressures are recorded at the beginning of each analysis and compared to previous readings to detect changes that indicate system problems.

10.0 Data analysis

10.1 Identification of analytes

10.1.1 Identification of analytes is done by comparing the sample retention times to standard retention times. Acceptable window sizes vary depending on the analytes. The early eluting compounds are more likely to shift during the course of the analysis. The later eluting analytes typically shift less. Shown below are typical retention times for the analysis. These may shift slightly during the course of a single analysis. The retention times may shift for the peaks, but their elution order will not change using the conditions of this

method. They may shift more significantly if a new column is used or other conditions are modified.

Typical carbamate retention times:

Analyte	Time(min)
Aldicarb sulfoxide	3.99
Aldicarb sulfone	4.98
Oxamyl	5.50
Methomyl	6.41
3-hydroxycarbofuran	12.13
Aldicarb	14.25
Carbofuran	16.49
Carbaryl	17.35

10.1.2 Typical chromatograms - Typical chromatograms are included at the end of this SOP.

10.2 Computer calculations

10.2.1 When the analysis has been completed, the standard chromatograms are reviewed to determine average retention times for each analyte. The active method is then updated (if necessary) with the new times.

10.2.2 Each chromatogram must be reviewed for appropriateness of integration. Adjustments are made, if necessary.

10.2.3 Once the analyst is satisfied that the chromatograms have been integrated appropriately, the reports are printed with the chromatogram and peak concentrations.

10.3 Manual calculations

10.3.1 Determine the response factor (Rf) for each analyte.

Rf = standard concentration/standard area

The Rf units are therefore the standard units, ng/ml.

Calculate the Rf for each analyte in each standard injection and then determine the average Rf for that analyte.

10.3.2 Determine the concentration© of any analytes in the samples.

C = sample area X Rf

The units of C are ng/ml which is equivalent to μg/L.
This is the result to be reported.

11.0 Documentation

11.1 Results are typically recorded in a notebook.

11.2 Compare the quality control samples to their control limits. The spike results are recorded

11.3 Computer files

11.3.1 The method file associated with the analysis and the analysis file, which includes the chromatogram and results are saved on tape backup.

11.4 Hard copies of the reports are filed.

12.0 References

12.1 EPA Method 531.1. Measurement of N-methylcarbamoyloximes and N-methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization, Revision 3.1 (Edited 1995).

12.2 Hill, K.M., R.H. Hollowell, and L.A. DalCortevo, "Determination of N-Methylcarbamate Pesticides in Well Water by Liquid Chromatography and Post Column Fluorescence Derivatization", Anal. Chem., 56, 2465 (1984).

12.3 "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979

12.4 Foerst, D.L. and H.A. Moye, "Aldicarb in Drinking Water via Direct Aqueous Injection HPLC with Post Column Derivatization", Proceedings of the 12th annual AWWA Water Quality Technology Conference, 1984.